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Short communication

Serum metalloproteinase 9 levels increase after generalized tonic-clonic seizures

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ABSTRACT

Metalloproteinase 9 (MMP9) is a member of a family of enzymes that mediate the degradation of extracellular matrix proteins, and is especially involved in blood–brain barrier maintenance. Increased levels of MMP9 have been observed in many neurological disorders, including epilepsy, suggesting it may be involved in the pathogenesis of seizures. We investigated changes in MMP9 serum levels after acute seizures in epilepsy patients. Concentrations of MMP9 in serum were measured by ELISA in 43 patients 1–3, 24, and 72 h after generalized tonic-clonic seizure and once in participants of the control group. MMP9 levels were significantly increased 1–3 and 24 h after seizure and decreased to control levels 72 h after seizure. Our results suggest that MMP9 is released after or just before seizure; however, further studies are needed to resolve the consequences of the observed MMP9 increase.

1. Introduction

Experimental and clinical data suggest that inflammation in the central nervous system (CNS) plays a critical role in epileptogenesis and in prompting seizures. CNS inflammation can activate microglia and astrocytes, with consequent damage to the blood–brain barrier (BBB) (Vezzani and Granata, 2005). Increased permeability of the BBB to leukocytes, albumin, and other factors leads to intensification of inflammation and hyperactivity of neurons (Van Vliet et al., 2007). Moreover, excessive bioelectric discharges and seizures stimulate BBB leakage and glial activation and promote inflammation. Thus, inflammation and seizures form a vicious circle, leading to development of epilepsy, possible drug resistance and tissue damage (Vezzani and Granata, 2005).

Metalloproteinase 9 (MMP9, gelatinase B) is a proteolytic zinc-dependent enzyme involved in many processes in the brain (Dziembowska and Wlodarczyk, 2012; Wilczynski et al., 2008). MMP9 is involved in the degradation of components of the basal lamina around capillaries, which permits the passage of leukocytes through the BBB to the inflammatory foci. MMP9 also facilitates angiogenesis and neurogenesis (Rosenberg, 2009).

MMP9 is found in the brain, especially the hippocampus and cerebral cortex (Dziembowska and Wlodarczyk, 2012). MMP9 levels are increased in many neurological disorders of inflammatory etiology or those affecting endothelium, such as multiple sclerosis and stroke.

Based on the proposed role of neuroinflammation in the pathogenesis of epilepsy and the possible involvement of BBB leakage in seizures, we investigated if levels of MMP9 changed over time following seizure by measuring MMP9 serum levels in patients with epilepsy after seizures and comparing them with those of control-group participants.

2. Materials and methods

The study was approved by the Committee for Ethics in Human Research at the Institute of Psychiatry and Neurology in Warsaw, Poland.

2.1. Patients

The study was performed in the Second Department of Neurology, Institute of Psychiatry and Neurology. The 43 patients were recruited from among patients with epilepsy hospitalized in the department after single (one to three) tonic-clonic seizures.

Inclusion criteria for the experimental group were as follows: age >18 years, diagnosis of focal or generalized epilepsy or first-ever

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generalized tonic-clonic seizure confirmed by a reliable witness and signed written consent of the patient. The control group comprised of nonepileptic adult patients in the department and healthy volunteers, who signed written consent.

Exclusion criteria for both the control and experimental groups included malignant tumor, concomitant inflammatory disease, severe neurological or neuroimmunological disease (i.e. acute stroke, cerebral hemorrhage, encephalitis, meningitis, or multiple sclerosis), immunosuppressive or immunomodulatory treatment during the previous 6 months, surgery or significant trauma within the previous 2 weeks, hepatic or renal insufficiency, severe psychiatric disease or pregnancy, and symptoms of infection.

2.2. Blood collection

Blood was collected three times from patients in the experimental group: 1–3 h, 24 h, and 3 days after seizure. Blood was collected once from patients in the control group. The venous whole blood sample (10 ml) was immediately centrifuged, and the serum was collected and frozen at −80°.

2.3. MMP9 measurement

MMP9 serum levels were measured with sandwich-type ELISA in accordance with the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). Absorbance at 450 nm was measured with the Multiskan Go (Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Statistical analysis

One-way analysis of variance with repeated measures was used to analyze the medical characteristics of the patients and MMP9 levels at different time points after the seizure. The Newman–Keuls test was used for individual post-hoc comparisons. For comparisons of means between two groups, Student's *t*-test was used. Pearson's correlation test was applied to estimate correlations between age and MMP9 levels. Significance was assumed at $p < 0.05$. The Statistica 12.0 software package for Windows (StatSoft, Tulsa, OK, USA) was used to analyze all data.

3. Results

We examined 43 patients who had been hospitalized after tonic-clonic seizures (19 women and 24 men) and 43 patients from the control group, matched for sex and age. The mean (±range) age of the epilepsy and the control group was 42,9 ± 17.8 years and 43,2± 16.8 years, respectively (Table 1). The mean age of female patients in the both groups was slightly, but insignificantly lower than that of male patients (Table 1). The group of patients who had seizures consisted of patients with various types of epilepsy histories: 23 had newly diagnosed epilepsy, 12 had a 1–10 year history of epilepsy, and eight had disease duration >10 years. The etiology of epilepsy included generalized idiopathic epilepsy (five cases), symptomatic epilepsy (21 cases), and epilepsy of unknown origin (17 cases). All of the patients had 1–3 generalized tonic-clonic seizures just before hospitalization (confirmed by eyewitness, mainly medical staff); status epilepticus and any other type of seizure were not included.

Patients qualified to participate in the study had no clinical signs of infection; WBC counts were <10,000/mm^3, and high-sensitivity C-reactive protein levels were <10 ng/ml.

The mean serum MMP9 level of the control group was treated as a reference value. MMP9 levels were significantly increased 1–3 and 24 h after seizures. By 1–3 and 24 h after seizures, MMP9 levels had reached an approximately 2-fold higher value and approximately 1.5-fold higher value, respectively, compared to the control level (Fig. 1.). MMP9 levels had decreased to the control level 72 h after seizure ($p < 0.142$). The mean MMP9 level was similar between men and women in the control group and in the epilepsy group at every time points (Table 1). MMP9 levels were correlated with age in the control group, increasing in older patients; however, we did not observe this effect in the epilepsy group at any time point (Fig. 2). MMP9 levels in the epilepsy group did not differ depending on etiology of epilepsy (symptomatic vs idiopathic).

4. Discussion

MMP9 serum levels increased just after seizure; this increase lasted for about a day and returned to normal levels after 3 days. We propose that the increased MMP9 levels are triggered by seizures and are a consequence of BBB disruption. Increased MMP9 levels were found previously in cerebrospinal fluid (CSF) 24 h after acute seizure. This increase was correlated with BBB permeability, as measured by the albumin CSF/blood ratio (QAlb) (Li et al., 2013).

MMP9 is associated with the pathology of many neurological diseases, including stroke, cancer, multiple sclerosis, and CNS infections. MMP9 levels may be related to the outcome or affect the pathogenesis of various CNS disorders. Increased MMP9 levels are correlated with the magnitude of brain damage, for example, in stroke and neurolupus, and indicate BBB disruption (Van Vliet et al., 2007). There is also evidence that MMP9 levels are increased in serum or in CSF in epilepsy; however, it is unclear whether this is associated with seizure development. For example, in infants with HHV6 infection, higher MMP9 levels are correlated with the emergence of febrile seizures, suggesting that MMP9 may be involved in inducement of seizures (Kittaka et al., 2014). MMP9 serum levels are also higher in patients with neurocysticercosis complicated by seizures compared to patients with comparable brain infection but without seizures (Rosell and Lo, 2008). In bacterial meningitis, MMP9 may be a risk factor for the development of neurological complications and seizures (Leppert et al., 2000).

Increased levels of MMP9 may be induced by seizures themselves. In experimental models of epilepsy induced by kainic acid or pentylenetetrazol, MMP9 levels and activity are increased after seizures. The main source of MMP9 may be leukocytes, since their number commonly increases after seizures (Ravizza et al., 2008). However, MMP9$^{-/-}$ transgenic mice show less epileptic activity after administration of pentylenetetrazol (Wilczynski et al., 2008). Inhibitors of MMP9 also prevent generation of seizures (Pollock et al., 2014). MMP9 is involved in the pathological process of epileptogenesis. It is highly expressed in hippocampus in patients with temporal lobe epilepsy and hippocampal sclerosis (TLE-HS)(Heuser et al., 2010).

We found that MMP9 levels increased with age in the control group, as observed previously (Simson et al., 2013). However, we did not observe an association between MMP9 levels and age in the epilepsy group. The increases of MMP9 after seizures were comparable in men and women at every time point.

4.1. Conclusion

After generalized tonic-clonic seizures, MMP9 levels increased in as quickly as few hours after the event. This increase remained observable 24 h after seizure and then decreased to near control levels by 72 h. Increased levels of MMP9 may be an indicator of increased BBB permeability and MMP9 involvement in pathological processes following seizures. MMP9 levels are correlated with the occurrence of seizures in brain infection and injury; thus, it would be worthwhile to investigate in future studies whether MMP9 serum levels are associated with the severity of seizure and risk of recurrent seizure (Ichiyama et al., 2007).